(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 March 2004 (18.03.2004)

PCT

(10) International Publication Number
WO 2004/023148 A1

(51) International Patent Classification[7]: G01N 33/74, 33/573, 33/68

(21) International Application Number:
PCT/FI2003/000653

(22) International Filing Date:
5 September 2003 (05.09.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
20021598 6 September 2002 (06.09.2002) FI

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(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT (utility model), PT, RO, RU, SC, SD, SE, SG, SK (utility model), SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR DETECTING A RISK OF ACID RELATED DISEASE IN AN INDIVIDUAL

(57) Abstract: The present invention is directed to a method for detecting a risk of gastric acid related disease in an individual based on assaying the analytes pepsinogen I, fasting gastrin-17 and a marker for *Helicobacter pylori* infection (Hp-marker), the method comprising selecting a cut-off value for pepsinogen I, fasting gastrin-17 and the Hp-marker, determining the concentration of pepsinogen I, fasting gastrin-17 and the concentration or presence of a Hp-marker in a sample from said individual, andcomparing the concentrations so determined with the selected cut-off values, whereby a pepsinogen I value at or above its respective cut-off value in combination with a Hp-marker value below its respective cut-off value and a fasting gastrin-17 value at or below its cut-off is indicative of an increased risk of gastric acid-related disease in said individual.

WO 2004/023148 A1

METHOD FOR DETECTING A RISK OF ACID RELATED DISEASE IN AN INDIVIDUAL

FIELD OF THE INVENTION

The present invention is directed to a method for detecting a risk of gastric acid related disease in an individual, in particular, a method for detecting a risk of gastro-esophageal reflux disease and Barrett's esophagus with associated risk of adenocarcinoma. The method can also be used in order to determine the risk for an individual to develop ulcers as a consequence of NSAID (non-steroidal anti-inflammatory drug) use, as well as a risk of developing esophageal disease after *Helicobacter pylori* eradication treatment. The method according to the invention is based on assaying the analytes pepsinogen I, a marker for *Helicobacter pylori* and fasting gastrin-17 from a sample, especially a serum sample, of said individual.

BACKGROUND OF THE INVENTION

Reflux disease is a condition that can be a consequence of the contents of the stomach rising, refluxing, into the esophagus. Specifically the term reflux disease is used when the said reflux phenomenom causes damage or an inflammation in the mucus of the esophagus, e.g. esophagatis. Typical symptoms of reflux disease are heartburns.

A complication of extended reflux disease is a disease called Barrett's esophagus, a disease involving epithelial changes of various degrees in the esophagus. It is believed that reflux destroys the epithelium of the esophagus causing a condition that in turn involves an increased risk of adenocarcinoma of the esophagus.

The stomach of an individual with a healthy, that is a non-atrophic mucosa is acidic, normally at a pH of appr. 1 to 2. The acid excretion in the stomach is however tied to

the release of gastrin-17, in that gastrin-17 stimulates the release of acid and an increase in the acidity of the stomach leads to a reduction of the release of gastrin-17 from antral G-cells (gastrin cells) in a feed back mechanism and control. This gastrin-17 is especially the gastrin-17 obtained at a basal or fasting situation, because the gastrin-17 which is measured after stimulation, such as after intake of a protein rich meal, increases, due to the neutralizing effect of the meal on the acidity of the stomach.

The publication WO 96/15456, which is included herein for reference, discloses a method for screening for the risk of cancer by determining the concentration of the analytes pepsinogen I and gastrin-17 from a serum sample of a subject. According to the said publication, the so determined concentration values are then compared to a selected cut-off value and a reference value for each analyte and the results so obtained can be used to evaluate the presence and location of atrophy in the stomach and associated risk of cancer.

According to an embodiment disclosed, the said tests may be combined with a test for *Helicobacter pylori* antibodies as well as a so-called protein stimulation test, according to which a blood sample is taken in the morning after fasting, whereafter the patient eats a protein-rich standard meal and blood samples are taken at 15 minute intervals for two hours. The maximal increase in gastrin-17 is evident after appr. 20 minutes.

Helicobacter pylori is a spiral shaped, gram-negative bacterium which thrives in the mucus in the immediate vicinity of the surface epithelial cells of the gastric mucosa and in the cell interstices. The bacterium apparently is transmitted perorally from one person to the other. The effect of the bacterium on the gastric mucosa is an inflammation reaction, which is mediated over a complement by liberating strong inflammation mediator substances. After the acute stage, the inflammation is transformed into chronic gastritis. In patients suffering from chronic gastritis, in 70 to 90 % a

Helicobacter pylori infection can be established (Calam, J (1994) Helicobacter pylori (Review) Eur. J. Clin Invest 24: 501-510).

The WO-publication WO 00/67035, which is included herein for reference, discloses a method for assessing the risk of peptic ulcer by determining quantitatively the concentration of serum pepsinogen I and serum gastrin-17 and comparing the obtained values to selected cut-off and reference values. The results so obtained makes it possible to evaluate a possible increased risk of peptic ulcer.

Methods are known in the art for measuring the concentrations of the various analytes, and there are also commercially available kits for this purpose. Some exemplatory methods for carrying out the said determinations are described in the WO-publication 96/15456 as well.

SUMMARY OF THE INVENTION

According to the invention it has now been discovered that it is possible to detect a risk of gastric acid related diseases in an individual, by determining the analytes pepsinogen I, a marker for *Helicobacter pylori* infection and gastrin-17, which is specifically gastrin-17 obtained in a fasting situation. If it based on the pepsinogen I and Hp-marker determinations can be established that the said individual has a non-atrophic and non-gastritic (no *H. pylori* infection) mucosa of the stomach, i.e. the pepsinogen I value is above the cut-off value indicating a healthy corpus mucosa, and the individual does not have a *Helicobacter pylori* infection, for example by establishing a low *Helicobacter pylori* antibody titer, a low fasting gastrin-17 value is indicative of an over-acidic stomach and an increased risk for the individual to develop acid related disease, such as those referred to above.

In the work leading up to the invention it could be established that a low fasting gastrin-17 (G-17fast) level in serum (<1 pmol/l) indicates a highly acidic stomach (acid output

is constantly high) which intragastric H^+ concentration inhibits the release of gastrin-17 from the antral gastrin cells in the stomach. Serum levels of gastrin-17 is an indirect marker of the gastric acidity in subjects in whom the gastric mucosa is normal and healthy, i.e. no *Helicobacter pylori* antibodies and serum pepsinogen I is normal.

It has been established with tests that low fasting gastrin-17 in serum indicates at least a 2fold increase of the risk of acid related diseases, of which can be mentioned e.g., gastroesophageal reflux disease (including those with cardia ulcers and polyps), Barrett's esophagus, duodenal ulcer disease that is not related to *H.pylori* infection, and gastroesophageal reflux disease that develops after eradication of the *H.pylori* infection.

Specifically the present invention is directed to a method for detecting a risk of gastric acid related disease in an individual based on assaying the analytes pepsinogen I, fasting gastrin-17 and a marker for *Helicobacter pylori* infection (Hp-marker), the method comprising

- determining the concentration of pepsinogen I, fasting gastrin-17 and the concentration or presence of a Hp-marker in a sample from said individual, and
- comparing the values so determined with a selected cut-off value for each analyte, whereby a pepsinogen I value at or above its respective cut-off value together with a Hp-marker value below its respective cut-off value in combination with a fasting gastrin-17 value at or below its respective cut-off, is indicative of an increased risk of gastric acid-related disease in said individual.

Thus the present invention provides a method which makes it possible to identify an individual with an increased risk to develop an acid related disease, such as those exemplified above, as well as risk of associated adenocarcinoma.

The method as defined can thus be used as an indirect test for gastric acidity in an individual from a sample, such as a blood, serum or plasma sample.

DETAILED DESCRIPTION OF THE INVENTION

According to the invention, the process of comparing the measured value to a cut-off value of an analyte is aimed at establishing if the measured value of the analyte is greater than, equal to or smaller than the respective cut-off value.

In the context of the present invention, an individual or a subject means a mammal, such as a human, or an animal, such as a pet, e.g. a dog.

The present invention thus includes in a first step a method for determining at least a *Helicobacter pylori* marker, pepsinogen I (PGI) and fasting gastrin-17 in a sample from the individual to be tested.

The marker or indicator for *Helicobacter pylori* infection can thus, according to the invention, be for example a *Helicobacter pylori* antibody, the value of which can be measured from a body fluid sample. Such a sample is advantageously a serum sample, but can also be a saliva, urine or lacrimal fluid sample, e.g. commercial kits being available for measuring the antibody value. The cut-off value for *Helicobacter pylori* antibodies can easily be determined by a person skilled in the art. In one embodiment of this invention we have used a value of 30 EIU as a cut-off for indicating the presence or absence of a *Helicobacter pylori* infection. Specific antibodies to *Helicobacter pylori* can also be measured using western blot.

Another alternative is to evaluate the presence of a *Helicobacter pylori* infection by determining the presence of the antigen itself. Such measurement can for example be carried out on a stool sample from a patient, and assays, such as enzyme immuno-assays are commercially available for this purpose (cf. for example Lancet 1999; 354,

30-33). It is also possible to determine the presence of the antigen from the breath of a patient, using the well known techniques of measuring the carbon dioxide content, the formation of which from labeled urea is catalyzed by *Helicobacter pylori* bacteria, and systems for this assay are also commercially available (e.g. Heliprobe™ by Noster AB, Sweden). Another alternative is to determine the presence or absence of the antigen in a biopsy sample taken during endoscopy from the stomach of a patient. In this alternative the tests are either positive or negative for antigen, and a presence of antigen is taken as being indicative of a *Helicobacter pylori* infection. In the context of this invention, when the Hp-marker is the antigen itself, the test is positive or negative, and the cut-off for infection, being the border between these, can thus be considered to be zero, a positive test being indicative of presence of antigen and consequently the abnormal condition, and a negative test being a value below the cut-off for this marker.

Pepsinogen and gastrin are preferably determined from a body fluid sample, especially from a serum, plasma or whole blood sample, or from a urine, salivary, or lacrimal fluid sample.

The method includes measuring the pepsinogen I analyte, but according to an embodiment of the invention it is also possible the measure, in addition, the concentration of the pepsinogen II analyte (PGII), and using the ratio PGI to PGII instead of PGI, or in addition thereto as the value to be compared to a predetermined cut-off value. Pepsinogen I and also the ratio of pepsinogen I to II tend to decrease linearly with worsening of the atrophy of the corpus mucosa.

In the method according to the invention a cut-off value for pepsinogen I is used, which is the normal cut-off value for differentiating between normal and atrophic corpus mucosa. According to one embodiment of the invention which has given reliable results, a cut-off value for serum or plasma pepsinogen I of $\geq$ 50 µg/l has been used. This value is somewhat higher than an often used cut-off of 25 µg/l and it

can be used in order to ensure that the individual tested certainly has a non-atrophic mucosa. The upper reference limit for serum or plasma pepsinogen I is of the order of 130 μg/l, whereby a value in the vicinity of or above the upper limit can be used as a confirmation of an increased risk of disease. However, a person skilled in the art can easily choose the cut-off value to be used for a specific situation and a specific population to be tested.

Likewise, for gastrin, a cut-off value can be determined, and according to the invention fasting gastrin-17 is measured. According to one embodiment of the invention, a cut-off value of fasting gastrin-17 of $\leq$ 1 pmol/l has been found suitable for most purposes. Such a value has also proven to be effective in providing a differentiation between individuals at risk and individuals not at risk for developing and acid related disease as defined above.

The concept of cut-off values in assays involving the determination of analyte concentrations is well known to the person skilled in the art. The cut-off value generally means a value or a set of values chosen as a limit between the reference values (normal values) and the abnormal values for the test in question. Such cut-off values are method-specific and depend on the specifity and sensitivity chosen for the test method used for the determination of the analyte concentrations, see for example William J Marshall, Clinical Chemistry, Third Edition, 1995, Mosby.

As mentioned earlier, the methods for measuring pepsinogen I, optionally pepsinogen II and gastrin-17 are well known in the art. The measurements are usually immunological methods, using mono- or polyclonal antibodies to the said analytes.

The detection methods for use include, for example, measuring absorbance, fluoresence or luminescence. It is also possible to carry out all three analyte measurements simultaneously, for example on the same microplate, in different wells or in the same well thereon, which provides for an especially convenient method.

As mentioned earlier there are also commercial kits available for measuring the various analyte concentrations, such as the GastroPanel (Biohit Oyj, Helsinki, Finland) for measuring i.a. pepsinogen I, gastrin-17 and *Helicobacter pylori* antibodies from a blood sample. The measured data can then be assessed for example using software, such as the GastroSoft® software, which draws up a diagnosis and gives recommendation of further treatments based on the results obtained for the analyte concentrations.

DETAILED DESCRIPTION OF THE DRAWING

In the drawing,

Fig. 1 shows the level of fasting gastrin-17 for Barrett negative and Barrett positive patients with normal stomach. From the results it can be seen that of the Barrett positive patients, 55% had a fasting gastrin-17 value equal to or less than 1 pmol/l, whereas only 23 % of the Barrett negative patients had such a low gastrin-17 value. The difference is statistically significant. Thus the fasting gastrin-17 is a marker distuingishing individuals at risk from individuals not at risk for developing acid related disease in a group of individuals with normal stomach.

Fig. 2 shows the level of fasting gastrin-17 for Barrett negative and Barrett positive patients with normal stomach, and

Fig. 3 the corresponding levels for postprandial gastrin-17 (stimulated gastrin-17). It is evident that fasting gastrin-17 gives a statistically significant differentiation between Barrett negative and Barrett positive patients, whereas the same is not true for postprandial gastrin-17.

Experimental tests

The results shown in Figs 1 to 3 are based on the following tests.

The material tested consisted of 300 endoscopied patients with gastrodyspepsia. They were prospectively collected and two groups of patients were formed. The cases composed of 15 patients with histologically and endoscopically verified classical long segment Barrett. Of these 11 had a normal and healthy gastric mucosa as defined by the absence of *H.pylori* gastritis (*H.pylori* IgG antibody titer <30 EIU) and normal (≥50 µg/l) serum level of pepsinogen I. They also had normal gastric histology in endoscopical biopsies from antrum and corpus. The controls (126 patients) composed of patients without Barrett's esophagus and *H.pylori* antibodies and with normal serum pepsinogen I. These two groups (cases and controls) were compared.

The mean fasting level of amidated gastrin-17 (G-17fast) was significantly (P=0.045 Mann-Whithey U test) lower in patients than in controls (4.0±6.1 pmol/l vs. 2.0±3.0 pmol/l). On the other hand, no differences were between cases and controls in postprandial G-17 levels (G-17prand). Instead, the G-17 prand was slightly higher in Barrett-patients than in controls (15.7±18.2 pmol/l vs 13.4±18.2).

When the 1 pmol/l was set to cut-off limit, 6 of the 11 Barrett patients (55%) had a G-17fast level below this cut-off whereas this prevalence was 29 of 126 (23%) among the controls (P<0.05; chi-square test).

There were, in addition, 5 patients with duodenal ulcer (DU) that occurred in association with a healthy, normal gastric mucosa (the etiology of ulcer in these patients is the usage of NSAIDs or aspirin, in addition to normal or high acid output). The serum level of G-17fast in these 5 DU patients was extremely low (0.458±0.431 pmol/l); i.e., significantly lower than that in appropriate non-DU, non-Barrett controls.

Claims

1. Method for detecting a risk of gastric acid related disease in an individual based on assaying the analytes pepsinogen I, fasting gastrin-17 and a marker for *Helicobacter pylori* infection (Hp-marker), the method comprising

- determining the concentration of pepsinogen I, fasting gastrin-17 and the concentration or presence of a Hp-marker in a sample from said individual, and
- comparing the values so determined with a selected cut-off value for each analyte, whereby a pepsinogen I value at or above its respective cut-off value together with a Hp-marker value below its respective cut-off value in combination with a fasting gastrin-17 value at or below its respective cut-off is indicative of an increased risk of gastric acid-related disease in said individual.

2. The method according to claim 1, wherein the *Helicobacter pylori* marker is a *Helicobacter pylori* antibody, the concentration of which is measured from the sample.

3. The method according to claim 1, wherein the *Helicobacter pylori* marker is the *Helicobacter pylori* antigen, the presence of which is determined in the sample.

4. The method according to any one of the preceding claims, wherein, in addition, the stimulated gastrin-17 value (G-17st), is measured.

5. The method according to any one of the preceding claims, wherein, in addition, the concentration of the analyte pepsinogen II (PGII) is measured, and the ratio PGI/PGII is formed for comparison.

6. The method according to claim 2, wherein the analytes are measured from a body fluid, such as a serum, plasma, whole blood, urine, saliva or lacrimal fluid sample, especially a serum sample.

7. The method according to any one of the preceding claims, wherein the gastric acid related disease is reflux disease or Barrett's esophagus.

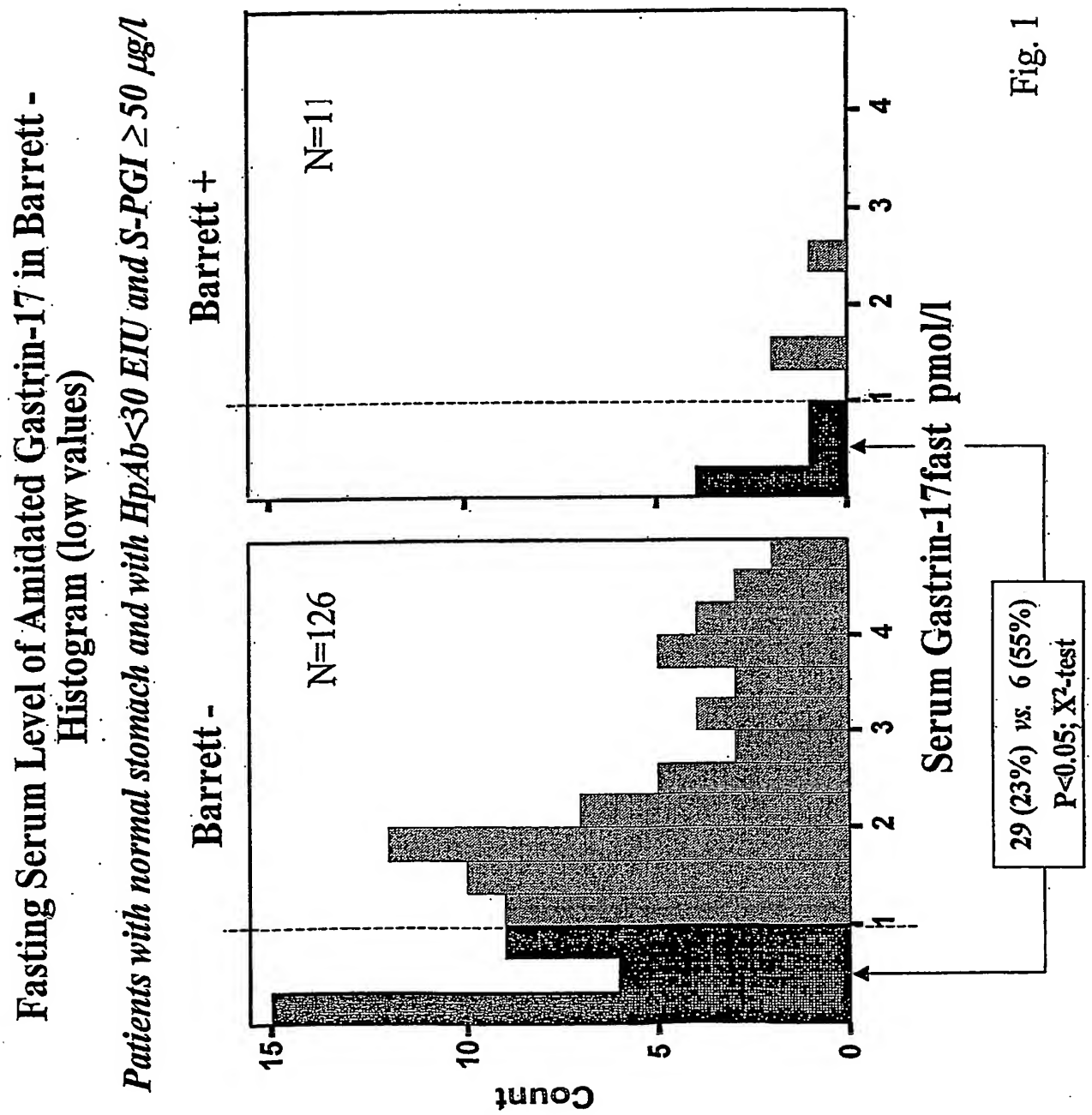
Fasting Serum Level of Amidated Gastrin-17 in Barrett -
Histogram (low values)
Patients with normal stomach and with HpAb<30 EIU and S-PGI ≥ 50 µg/l
Barrett -
N=126
Barrett +
N=11
Count
15
10
5
0
1
2
3
4
Serum Gastrin-17fast pmol/l
29 (23%) vs. 6 (55%)
P<0.05; X²-test

Fig. 1

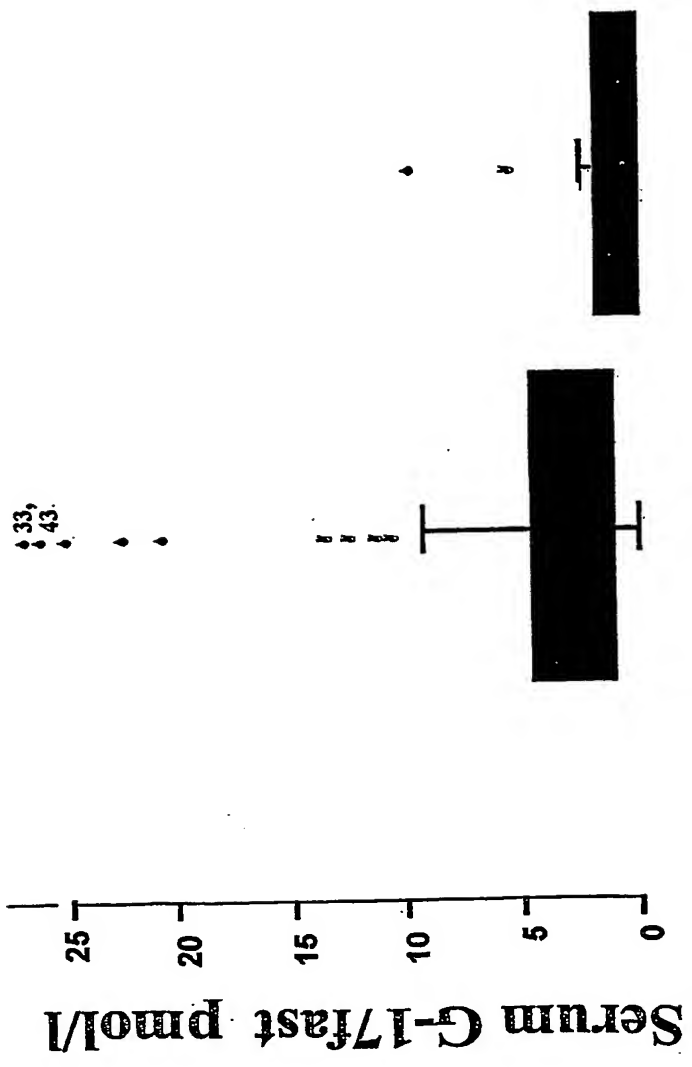
Fasting Serum Level of Amidated Gastrin-17 in Barrett
Patients with normal stomach and with HpAb<30 EIU and S-PGI ≥ 50 µg/l
Barrett -
Barrett +
Serum G-17fast pmol/l
25
20
15
10
5
0
33,
43
N= 126 11
Mean ±SD 4.0 ±6.1 2.0 ±3.0
Mann-Whitney U P=0.045

Fig. 2

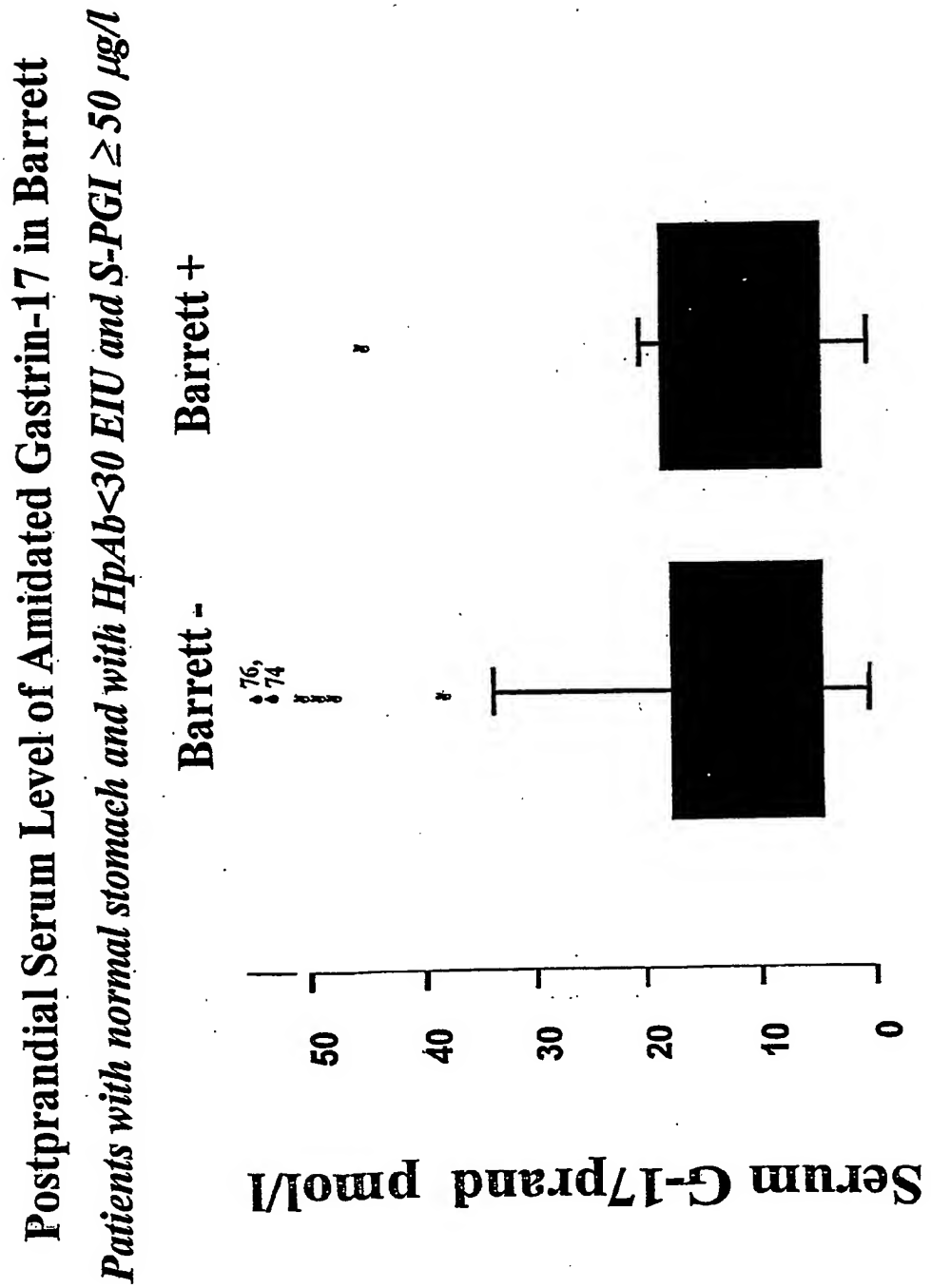
Postprandial Serum Level of Amidated Gastrin-17 in Barrett
Patients with normal stomach and with HpAb<30 EIU and S-PGI ≥ 50 µg/l
Barrett -
Barrett +
76,
74
50
40
30
20
10
0
Serum G-17prand pmol/l
N=
126
11
Mean ±SD
13.4 ±18.2
15.7 ±18.2
Mann-Whitney U
P=0.828

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 03/00653

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/74, G01N 33/573, G01N 33/68
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI DATA, PAJ, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	National Library of Medicine, (NLM), File Medline, Medline accession no. NLM7903552, Park S.M. et al, "G- and D-cell populations, serum and tissue concentrations of gastrin and somatostatin in patients with peptic ulcer diseases", & The Korean journal of internal medicine, Korea 1993, Vol. 8, No. 1, pg. 1-7, abstract --	1-6
Y	WO 02054084 A1 (BIOHIT OYJ), 11 July 2002 (11.07.02), page 7, line 1 - page 8, line 7; page 11, line 5 - line 14; page 11, line 19 - line 21 --	1-6

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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"P" document published prior to the international filing date but later than the priority date claimed
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"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 November 2003	03 -12- 2003
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer TERESE PERSSON/BS Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 03/00653

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Helicobacter, Volume 5, No. 1, 2000, Ken Haruma et al, "Negative Association Between Helicobacter pylori Infection and Reflux Esophagitis in Older Patients: Case-Control Study in Japan", pages 24-29 -- Gut, Volume 41, 1997, Y. Kinoshita et al, "Helicobacter pylori independent chronological change in gastric acid secretion in the Japanese", pages 452-458 -- --------	1-7 1-7

INTERNATIONAL SEARCH REPORT

Information on patent family members

06/09/03

International application No.
PCT/FI 03/00653

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 02054084 A1	11/07/02	EP 1348129 A	01/10/03
		FI 4986 U	18/07/01
		FI 20010019 A,V	06/07/02